

Practitioner's Docket No. MPI96-031CP1DV1CPACN2M

U.S.S.N. 10/810,793

RECEIVED
CENTRAL FAX CENTER

AUG 13 2007

IN THE SPECIFICATION

Please replace the paragraph at page 17, line 22, with the following paragraph:

FIG. 21. FIGS. 21A-21D are amino acid sequences for pep1-pep4 (SEQ ID NOs:3, 4, 5 and 6, respectively).

Please replace the paragraph at page 18, lines 1-2, with the following paragraph:

FIG. 22. FIGS. 22A-22B are nucleic acid sequences encoding p50 (SEQ ID NO:7) and p40 (SEQ ID NO:8), respectively.

Please replace the paragraph at page 85, line 6 to page 86, line 4, with the following paragraph:

cDNAs encoding, I κ B α and its mutants have been described (Brockman, J. A. et al., Mol. Cell. Biol. 15:2809-2818 (1995); Chen, Z. J. et al., *Genes & Dev.* 9:1586-1597 (1995)), ³⁵S-labeled I κ B α proteins were prepared by in vitro translation in wheat germ extracts (Promega). pGEX-2TK-UBCh5 was constructed by PCR using UBCh5 cDNA (provided by Dr. P. Howley) as a template. The active site mutants of UBCh5, pGEX-2TK-UBCh5 (C85A) and pGEX-2TK-UBCh5(C85S), were created by site-directed mutagenesis using the Unique Site Elimination (U.S.E) mutagenesis kit (Pharmacia). The mutagenic primers (anneal to noncoding strand) were: 5' TTG TGA CCT CAG GAT ATC GAG AGC AAT ACT TCC ATT 3' (SEQ ID NO:1) for C85A, and 5' TTG TGA CCT CAG GAT ATC. GAG AGA AAT ACT TCC AT 3' (SEQ ID NO:2) for C85S. All constructs were confirmed by DNA sequencing. For expression of GST-UBCh5 and its mutants, the appropriate expression constructs were transformed into the E. coli strain BL21/DE3, and protein expression was induced with 200 μ M IPTG. GST fusion proteins were purified using Glutathione-Sepharese SEPHAROSETM (Pharmacia GE Healthcare). Yeast UBC4 (yUBC4) was expressed in the E. coli AR58 harboring the UBC4 expression vector (pLAUBC4, provided by Dr. V. Chau). After heat induction (30° C to 42° C), UBC4 was purified by ubiquitin-Sepharese SEPHAROSETM (GE Healthcare) covalent chromatography, followed by gel filtration on FPLC/Superdex SUPERDEX[®]-200 (GE Healthcare). Recombinant RelA homodimer was prepared according to Thanos and Maniatis, *Cell* 80:529-532 (1992)(provided by Dr. J. Hagler). Purified recombinant human UBC2 was provided by Dr. O. Coux. Purification of E1, E2_{14K}, E2_{7K}, E2_{20K}, E2_{25K}, E2_{35K} from rabbit reticulocytes were according to Baas and

(Page 2 of 11)

Practitioner's Docket No. MPI96-031CP1DV1CPACN2M

U.S.S.N. 10/810,793

RECEIVED
CENTRAL FAX CENTER

AUG 13 2007

Bright, *J. Biol. Chem.* 263:13258-13267 (1988). Preparation of methylated ubiquitin and ubiquitin aldehyde have been described (Chen, Z. J. *et al.*, *Genes & Dev.* 9:1586-1597 (1995)). ¹²⁵I-ubiquitin was prepared by the Chloramine T method. Antibodies against IκBα and RelA were from Santa Cruz Biotechnology.

Please replace the paragraph at page 94, lines 6-13, with the following paragraph:

Purified IκBα kinase (from gel filtration chromatography) was treated with MEKK1Δ in 50 mM Tris (pH 7.6), 5 mM MgCl₂, 2 mM ATP for 30 min at 30° C. MEKK1Δ-activated IκBα kinase was separated from ATP by centrifugal gel filtration on ~~Sephadex~~ SEPHADEX® G50 (GE Healthcare) and subsequently incubated with or without calf intestinal alkaline phosphatase (CIP) in 50 mM Tris (pH 7.8), 0.1 mM EDTA for 30 min at 30° C. IκBα kinase was then separated from CIP and MEKK1Δ by chromatography on a ~~Superdex~~ SUPERDEX® 200 column (GE Healthcare) and assayed for IκBα kinase activity in the absence or presence of MEKK1Δ.